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(54) Process for the production of epigallocatechin gallate

(57) Epigallocatechin gallate (EGCG) is obtained by subjecting a green tea extract to chromatography on a macroporous polar resin; eluting EGCG from the resin with a polar elution solvent; optionally concentrating the eluate; optionally regenerating the resin by desorbing the remaining catechins; and optionally concentrating the desorbed catechins.

EP 1 077 211 A2

Description

[0001] The present invention relates to a process for the production of (-)-epigallocatechin gallate (EGCG). The invention particularly relates to a process for the production of EGCG by separation from tea catechins involving treatment with a macroporous polar resin.

[0002] Leaves of the green tea plant *camellia sinensis* contain up to 36% polyphenols on a dry weight basis, however, their composition varies with climate, season, variety and state of maturity. Green tea catechins are the predominant group of green tea polyphenols. Examples of catechins are (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), epigallocatechin (EGC) and epicatechin gallate (ECG).

[0003] EGCG is the most interesting compound among the above mentioned catechins because it exhibits a strong antioxidant effect. Furthermore, it has been demonstrated that EGCG has an antimutagenic effect, an antibacterial effect and also a beneficial effect on cholesterol level in blood. The other catechins present in tea are much less effective. Green tea also contains other components such as caffeine, proteins, pectins and/or metal ions which might not be desirable.

[0004] There is therefore a need to isolate EGCG in a pure form in high yield by a simple and economical process. However, the structural similarities of the various tea catechins make the separation of the individual catechins difficult. Furthermore, the catechins in tea are normally accompanied by caffeine being present in an amount up to 4% of the dry mass of the green tea leaves. Caffeine is known to associate with the catechins and is not trivial to remove.

[0005] The production of green tea extracts is well known in the art. US Patent No. 5,879,733 describes the preparation of a green tea extract having improved clarity and color. The tea extract is obtained by treating the green tea extract at a temperature in the range of 25° C to 60° C with an amount of a food grade cation exchange resin effective to remove metal cations present in the extract. The treated extract is then contacted with a nanofiltration membrane. However, the process described in US 5,879,733 is not suitable to separate EGCG from a mixture of tea catechins.

[0006] US Patent No. 4,613,672 describes a process for the preparation of pure EGCG which process comprises the following steps: Tea leaves are extracted with hot water or with aqueous solutions of 40-75% methanol, 40-75% ethanol or 30-80% acetone. The obtained extract is washed with chloroform and the washed extract is dissolved in an organic solvent. The organic solvent is distilled off, and the concentrated extract component is subjected to high speed liquid chromatography using a reverse-phase partition column with a developer of acetone/ tetrahydrofuran/water (0-25:0-35:65-85, vol%),whereby each of (-) epicatechin, (-) epigallocatechin, (-) epicatechin gallate and (-) epigallocatechin gallate is isolated from one another. The process described in US Patent No. 4,613,672 does not permit an economical production of EGCG in a technical scale because of the use of expensive column fillings. Furthermore the process described in US Patent No. 4,613,672 does not permit the production of EGCG which can be added to food products because the solvents mixture used (acetone/tetrahydrofuran/ chloroform) are not food-approved.

[0007] While the art describes the production of catechins as mixtures, there is still a need for a simple, safe and economic process for producing EGCG in a purified form for incorporation as an ingredient into supplements and foodstuffs.

[0008] It has now been found that EGCG can be separated from a mixture of tea catechins and/or caffeine with improved selectivity when carrying out the separation using a macroporous polar resin and a suitable polar elution solvent.

[0009] Thus, the present invention is directed to a process for producing epigallocatechin gallate (EGCG), which comprises the steps of

a) providing a green tea extract
b) subjecting the green tea extract to a chromatography on a macroporous polar resin at a temperature in the range of about 10° C to about 80° C
c) eluting EGCG from the macroporous polar resin with a polar elution solvent at a temperature in the range of about 10° C to about 80° C and at a pressure in the range of about 0.1 bar to about 50 bar;
d) optionally concentrating the eluate of step c);
e) optionally regenerating the macroporous polar resin by desorbing the remaining catechins; and
f) optionally concentrating the desorbed catechins of step e).

[0010] The production of the green tea extract used as starting material is well known in the art. For example green tea leaves are typically extracted with hot or cold water to form a solution containing tea catechins and caffeine . This green tea solution can be further concentrated to form either a concentrated extract solution or a dry powder. The extract solution or the powder can contain stabilizers such as food-approved acids, e.g. citric acid, ascorbic acid, isoascorbic acid and the like.

[0011] Tea extract powders are also commercially available e.g. from Guizhou Highyin Biological Product Co., Guiyang, P. R. China, or Zhejang Zhongke Plant Technical Co. Ltd., Hangzhou, Zhejang, P. R. China.

[0012] The separation of EGCG is typically carried out by subjecting the green tea extract to a column filled with macroporous polar resin.

[0013] As used herein, "macroporous polar resins" refers to acrylic resins such as polyacrylates e.g. AMBERLITE®XAD-7 available from Rohm and Haas, Philadelphia, Pa. or polymethacrylates such as e.g. AMBERCHROM ® CG-71 available from Toso Haas or DIAION HP 2MG available from Mitsubishi Chem. Corp., Philadelphia, Pa; or polyamides such as Polyamide 11 available from Merck, Darmstadt, Germany; Polyamide 6 and Nylon 6,6 available from Fluka, Buchs, Switzerland (catalogue Nos. 02395 and 74712, resp.); Polyamide 12 (Grilamid L 25 natur, available from EMS Chemie, Domat, Switzerland;. or Polyvinylpyrrolidone P 6755 available from Sigma; or aromatic polyamides and polyesters.

[0014] The resin is preferably operated degassed and equilibrated with the elution solvent.

[0015] The process according to the invention is performed at temperatures in the range of about 10° C to about 80° C, preferably of about 40° C to about 60° C. Thermostatic control can e.g. take place by placing the column in a thermostatically controlled area e.g. a heating jacket.

[0016] The hydraulic pressure under which the mobile phase is passed through the column can be varied within wide limits. The mobile phase is preferably pumped through the column at a pressure of about 0.1 bar to about 50 bar, preferably at about 0.1 bar to about 20 bar, more preferably at about 0.1 bar to about 10 bar.

[0017] The mobile phase comprises a polar elution solvent which is a mixture of water and an organic solvent. As used herein, "organic solvent" refers to alcohols, such as methanol, ethanol, isopropanol and the like, and ketones such as e.g. acetone or esters such as ethylacetate or mixtures thereof. The use of food grade alcohols, such as ethanol and isopropanol, is preferred. Particularly good results are obtained when using a mobile phase comprising a mixture of about 70 to about 95 vol%, preferably about 90 vol%, of water and about 5 to about 30 vol%, preferably about 10 vol%, of organic solvent. It is advantageous to degas and the mobile phase and keep it under an inert atmosphere such as e.g. nitrogen or argon.

[0018] The column is conditioned with the mobile phase. The flow rate of the mobile phase through the column can be varied within wide limits . The flow rate is in the range of about 0.5 to about 20 bed volumes/h, preferably about 0.5 to about 10 bed volumes/h, more preferably of about 0.8 to about 5 bed volumes/h. (1 bed volume corresponds to 1 m^3 solution or solvent per m^3 resin).

[0019] After the equilibrium has been established between the stationary and mobile phases, tea extract solution is introduced into the mobile phase, thus, subjecting the green tea extract to a chromatography on the macroporous polar resin. If a green tea extract powder is used as the starting material, the powder is dissolved in the mobile phase. If an aqueous green tea extract is used, it is advantageous to adjust the ratio of water to organic solvent in the extract to that of the mobile phase by adding organic solvent.

[0020] A key aspect of the present invention is to treat the green tea extract with a macroporous polar resin at temperatures in the range of about 10° C to about 80° C, preferably at about 40° C to about 60° C and eluting EGCG with a polar elution solvent. This particular interplay of the three features of resin, eluent and temperature forms an important aspect of the present invention and leads to a specific separation of EGCG from a mixture of tea catechins and/or caffeine, thus obtaining, after elution an EGCG fraction containing at least 75 % , preferably more than 85%, more preferably about 90 % to about 97 %, of EGGG calculated on the total amount of catechins present in the extract or concentrate.

[0021] The ability of the macroporous polar resin to absorb caffeine, EGCG and the remaining catechins is different depending also on the eluent used and the temperature. The affinity for caffeine is less than that of EGCG, thus, caffeine, if present, is eluted first and can be separated off. If appropriate it is also possible to recover caffeine thereby in a purified form which could be an economic advantage, too. A second fraction is isolated in which the EGCG is present. The remaining tea catechins show a stronger affinity than that of EGCG, thus, remaining adsorbed until the resin is regenerated by using a solvent which is able to desorb the remaining catechins. For example, the remaining catechins can be desorbed by eluting with a pure organic solvent or by changing the ratio of water to organic solvent in the mobile phase. A suitable regeneration solvent is e.g. a pure organic solvent or a mixture of about 10 vol% to about 60 vol% of water and of about 40 vol% to about 90 vol% of organic solvent, preferably about 40 vol% of water and about 60 vol% of organic solvent.

[0022] The concentration of the EGCG in the eluate can be carried out by methods well known in the art, e.g. by evaporation. The EGCG eluate can be evaporated to dryness to form a powder containing EGCG in high purity or concentrated to allow crystallization. The concentration can be carried out by adding a stabilizer to the eluate such as a food-approved acid, e.g. citric acid, ascorbic acid, isoascorbic acid and the like. The acid is preferably added in an amount of about 0.1 to about 2.5 % with respect to EGCG.

[0023] The pre-fraction containing the caffeine and the fraction of step e) containing the remaining catechins can be concentrated as described above.

[0024] The process can be carried out using a single column or a system of multiple chromatographic columns. The process can also be carried out using technologies of the art referred to in the art as "simulated moving bed chroma-

tography" or "annular chromatography".

[0025] The process of the present invention can be conducted with simple and economical operations and is thus applicable to a large scale production in respect of yield and handling.

[0026] EGCG prepared as described above possesses a strong antioxidant activity and can thus be used as an antioxidant for various foodstuffs, cosmetics, oils and so forth. In addition, EGCG has an antimutagenic effect, an antibacterial effect and also a beneficial effect on cholesterol level in blood. Thus, concentrates or pure EGCG are useful in health care preparations.

[0027] The present invention is illustrated in more detail with reference to the following examples.

Example 1 Separation of EGCG

[0028] A green tea extract containing the different catechins and caffeine (manufactured by Guizhou Highyin Biological Products Co., Guiyang, China as "Green Tea Extract, min 95 % of polyphenols") was used as starting material. The concentration of the tea components was determined by HPLC using UV absorbance and expressed as wt.-%. The content of EGCG, caffeine, other catechins as well as gallic acid is shown in Tab. 1.

Tab. 1

Concentration of the tea components in the starting material		
Compound	Tea extract Example 1 HPLC /wt.-%	Tea extract Example 1 Relative Percentage /%
Gallic acid	0.01	0.0
Catechin	2.3	3.2
Caffeine	11.0	15.1
EGCG	38.1	52.3
Epicatechin	5.2	7.1
GCG	6.6	9.1
ECG	9.7	13.3
Total	72.9	100.0

[0029] 33.5 l (26 kg) of Amberlite® XAD-7 resin having a particle size of 0.3 to 1.2 mm were filled into a pilot scale column having an inner diameter of 150 mm, a length of 2 m and a volume of 35.4 l. The column was equipped with a heating jacket. The resin was thoroughly washed with water and equilibrated with a mixture of water/isopropanol (ratio 9:1 by volume). The apparatus and the solvents used were degassed and kept under an inert nitrogen atmosphere prior to use.

[0030] The filled column was thermostated to 60° C. 0.4 kg of the above green tea extract (Tab. 1) containing 152.5 g of pure EGCG were dissolved in 1.8 kg of a mixture of water/ isopropanol (ratio 9:1 by volume) and applied to the top of the column. EGCG was eluted from the column by means of a pump under a pressure of 0.5 bar and a temperature of 60° C with a mixture of water/isopropanol (ratio 9:1 by volume) at a constant flow rate of 50 kg/h. After an initial eluate of 144 kg (prefraction), a main eluate of 174 kg was collected containing 112g of EGCG as the main polyphenol component. The EGCG concentration in the main eluate was 0.064 %. The yield of separated EGCG starting from 152.5 g EGCG in the tea extract was 73.5%.

[0031] To regenerate the resin, the remaining catechins were desorbed by eluting with 78.3 kg of a mixture of water/isopropanol (ratio 4:6 by volume). Before the next separation, the column was conditioned with 86 kg of a mixture of water/isopropanol (ratio 9:1 by volume) in backwash mode at a flow rate of 120 kg/h.

[0032] Table 2 illustrates the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the main eluate was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 2

Concentration of the tea components in the main eluate		
Compound	Main fraction Example 1 HPLC /ppm	Rel. Percentage /%
Gallic acid	0	0.0
Catechin	21	3.0
Caffeine	1	0.1
EGCG	644	92.1
Epicatechin	29	4.1
GCG	3	0.4
ECG	1	0.1
Total	699	100.0

Example 2

[0033] Example 1 was repeated using another lot of the "Green tea extract, min. 95% of polyphenols" from Guizhou Highyin Biological Products Co. which was composed as shown in Table 3.

Tab. 3

Concentration of the tea components in the starting material		
Compound	Tea extract Example 2 HPLC /wt.-%	Tea extract of Example 2 Rel. Percentage /%
Gallic acid	0.1	0.1
Catechin	1.4	1.9
Caffeine	13.8	18.8
EGCG	*35.1*	*47.9*
Epicatechin	3.3	4.5
GCG	8.2	11.2
ECG	11.4	15.6
Total	*73.2*	*100.0*

[0034] The washed column of Example 1 was thermostated to 60° C and used to carry out the separation. 0.4 kg of the above green tea extract (Tab. 3) containing 140.5 g of pure EGCG were dissolved in 1.8 kg of a mixture of water/isopropanol (ratio 9:1 by volume) and applied to the top of the column. The column was then eluted as described in Example 1. After an initial eluate of 200 kg (prefraction) a main eluate of 117 kg was collected containing 72.8 g of EGCG as the main polyphenol component. The EGCG concentration in the main eluate was 0.062 %. The yield of separated EGCG starting from 140.5 g of EGCG in the tea extract was 51.8%.

[0035] To regenerate the resin, the remaining catechins were desorbed by eluting with 100. kg of a mixture of water/isopropanol (ratio 4:6 by volume). Before the next separation step, the column was conditioned with 100 kg of a mixture of water/isopropanol (ratio 9:1 by volume) in backwash mode at a flow rate of 120 kg/h.

[0036] Table 4 illustrates the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the main eluate was determined by HPLC using UV absorbance and expressed as ppm. Compared to Example 1, EGCG was obtained in a higher percentage.

Tab. 4

Concentration of tea components in the main eluate.		
Compound	Main fraction Example 2 HPLC /ppm	Rel. Percentage /%
Gallic acid	0	0.0
Catechin	10	1.6
Caffeine	1	0.2
EGCG	*622*	*96.4*
Epicatechin	2	0.3
GCG	6	0.9
ECG	4	0.6
Sum	*645*	*100.0*

Example 3 Concentration of the eluate.

[0037] 9008 kg of the eluate of the adsorption/desorption column prepared by repeated runs as described in Example 2 were stabilized by addition of 2% citric acid, calculated on the EGCG amount. The eluate was concentrated at a temperature of 40 °C and a pressure of 55 mbar using a falling film evaporator made from stainless steel with a heat exchange surface area of 1.1 m^2. The amount of catechins and caffeine of the feed solution subjected to the evaporation unit is given in Table 5.

Tab. 5

Concentration of the tea components in the purified EGCG solution subjected to evaporation.		
Compound	Feed falling film evaporator HPLC /ppm	Rel. Percentage /%
Gallic acid	0	0.0
Catechin	10	1.4
Caffeine	1	0.1
EGCG	712	96.2
Epicatechin	7	0.9
GCG	7	0.9
ECG	3	0.4
Total	740	100.0

[0038] The feed flow to the evaporator was regulated to a flow rate in the range of 120 kg/h to 130 kg/h at a recycle flow rate of 300 kg/h. Thus, the distillate flow rate was 123.5 kg/h at a bottom product removal rate of 0.52 kg/h. During the concentration process a first fraction was sampled and analyzed, followed by a second fraction which was analyzed, too. The two fractions of EGCG concentrates had a total mass of 63.5 kg.

[0039] Tab. 6 shows the concentration of the tea components in the bottom products. The recovery of EGCG was 95.9 %. The analytical result clearly indicates that the high purity of the separated EGCG could be maintained during the concentration of the solution.

[0040] EGCG can be isolated from the concentrated solution in a solid form either by spray drying or by crystallization.

Tab. 6 Concentration of the tea components in the EGCG concentrate from the falling film evaporator.

Compounds	Bottom product of falling film evaporator			
	Composition 1st fraction		Composition 2nd fraction	
	HPLC /wt.-%	Rel. Percentage	HPLC / wt.-%	Rel. Percentage
Gallic acid	0.00	0.0	0.00	0.0
Catechin	0.17	1.5	0.14	1.6
Caffeine	0.01	0.1	0.02	0.2
EGCG	10.70	95.4	8.07	94.8
Epicatechin	0.16	1.4	0.11	1.3
GCG	0.13	1.2	0.13	1.5
ECG	0.05	0.4	0.04	0.5
Sum	11.22	100.0	8.51	100.0
Total mass of solution	39.0 kg		24.5 kg	

Example 4

[0041] 450 ml of AMBERCHROM® CG-71c having a mean particle diameter of 120 microns were filled into a laboratory chromatography column made from stainless steel having an inner diameter of 2.2 cm and a length of 103 cm. The column was equipped with a heating jacket. The resin was washed and equilibrated with a mixture of water/ethanol (ratio 9:1 by volume)

[0042] 20 g of a concentrated catechin powder "Green tea extract, min. 95% of polyphenols" from Guizhou Highyin Biological Products Co. (starting material) were dissolved in 20 ml of a mixture of water/ethanol (ratio 9:1 by volume). Afterwards, 14 g of this solution (corresponding to 2.99 g of EGCG) were applied to the top of the column. EGCG was eluted by means of a chromatographic pump under a pressure of 2-3 bar at a temperature of 60 °C with a mixture of water/ethanol (ratio 9:1 by volume) under a constant flow rate of 16 ml/min. The eluent was degassed and kept under an nitrogen atmosphere prior to use. After an initial eluate of 2.48 l (prefraction), the flow rate was changed to 25.5 ml/min and the main eluate of 5.40 l was collected containing EGCG in a concentration of 0.627 g/l. With respect to other catechins and caffeine, the purity of the EGCG in the main eluate determined by HPLCand expressed as relative percentage was 97.13 %. During the experiment, the pressure in the system varied from 2 to 3 bar depending on the flow rate applied. Tab. 7 compares the concentration of the tea components in the eluate and in the starting material, thus illustrating the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the starting material and in the main fraction was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 7

Separation on AMBERCHROM CG-71c, 60 °C, solvent system: water/ethanol				
Compound	Tea Concentrate (starting material) Example 4		Main Fraction Example 4	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
Gallic acid	0.08	0.1	0	0.0
Catechin	0.50	0.6	1	0.2
Caffeine	9.29	11.3	5	1.2
EGCG.	42.23	51.4	407	97.1
Epicatechin	4.24	5.2	3	0.7
GCG	8.09	9.9	1	0.2
ECG	17.70	21.6	2	0.5
Total	82.13	100.0	419	100.0

Example 5

[0043] 450 ml of Amberlite® XAD-7, having a particle diameter of 0.3 to 1.2 mm was packed into a laboratory chromatography column made of glass having an inner diameter of 2.4 cm and a length of 100 cm. The column was equipped with a heating jacket and on the bottom with a glass sinter frit P3. The resin was thoroughly washed with deionized water and equilibrated with a mixture of water/ethanol (ratio 9:1 by volume) prior to use.

[0044] 20 g of a concentrated catechin powder "Green tea extract, mm. 95% of polyphenols" from Guizhou Highyin Biological Products Co. (starting material) were dissolved in 20 ml of a mixture of water/ethanol (ratio 9:1 by volume). Afterwards, 14 g of this solution (corresponding to 2.91 g of EGCG) were applied to the top of the column. EGCG was eluted with a mixture of water/ethanol (ratio 9:1 by volume) with a constant flow rate of 16.9 ml/min at a temperature of 60° C and a pressure of 0.5 to 1 bar. The eluent was degassed and kept under an nitrogen atmosphere prior to use. After an initial eluate of 2.48 l (prefraction), the flow rate was changed to 23.6 ml/min and the main eluate of 4.95 l was collected containing EGCG in a concentration of 0.470 g/l. With respect to other main catechins and caffeine, the purity of the EGCG in the main fraction determined by HPLC was 86.1 % (see Tab. 8). The yield based on EGCG was 79.8 %. During the experiment, the pressure in the system varied from 0.5 to 1 bar depending on the flow rate applied.

[0045] To regenerate the resin, the remaining catechins were desorbed by eluting with 1.35 l of a mixture of water/ethanol (ratio 4:6 by volume) at a flow of 22.5 ml/min. This fraction can also be used for further purification or separation of the desorbed catechins. Table 8 compares the concentration of the tea components in the eluate and in the starting material, thus illustrating the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the starting material and in the main fraction was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 8

Separation on Amberlite XAD-7, 60 °C, solvent system: water/ethanol				
Compound	Tea Concentrate (starting material) Example 5		Main Fraction Example 5	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
Gallic acid	0.09	0.1	0	0.00
Catechin	0.50	0.6	2	0.4
Caffeine	9.17	11.5	7	1.3
EGCG	41.16	51.5	470	86.1
Epicatechin	4.16	5.2	5	0.9

Tab. 8 (continued)

Separation on Amberlite XAD-7, 60 °C, solvent system: water/ethanol				
Compound	Tea Concentrate (starting material) Example 5		Main Fraction Example 5	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
GCG	7.75	9.7	22	4.0
ECG	17.16	21.5	40	7.3
Total	79.99	100.0	546	100.0

Example 6

[0046] The regenerated resin of Example 5 was equilibrated in the laboratory column described in Example 5 by pumping a mixture of water/ethanol (ratio 9:1 by volume) through the resin.

[0047] 20 g of a concentrated catechin powder "Green tea extract, mm. 95% of polyphenols" from Guizhou Highyin Biological Products Co. (starting material) were dissolved in 20 ml of a mixture of water/ethanol (ratio 9:1 by volume). Afterwards, 14 g of this solution (corresponding to 3.04 g of EGCG) were applied to the top of the column. EGCG was eluted with a mixture of water/ethanol (ratio 9:1 by volume) with a constant flow rate of 22.5 ml/min at a column temperature of 40° C and a pressure of 1 to 2 bar. The eluent was degassed and kept under an nitrogen atmosphere prior to use. After an initial eluate of 3.60 l (prefraction) the flow rate was changed to a flow rate of 26.3 ml/min and the main eluate of 4.73 l was collected. The EGCG concentration in the main eluate was 0.278 g/l. With respect to other main catechins and caffeine the purity of the epigallocatechin gallate in the main eluate determined by HPLC was 93.2 %. The yield based on EGCG was 42.8 %. During the experiment the pressure in the system varied from 1 to 2 bar depending on the flow rate applied.

[0048] To regenerate the resin, the remaining catechins were desorbed by eluting with 1.98 l of a mixture of water/ethanol (ratio 4:6 by volume) at a flow of 26.3 ml/min and a temperature of 40° C. This fraction can also be used for further purification or separation of the desorbed catechins. Table 9 compares the concentration of the tea components in the eluate and in the starting material, thus illustrating the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the starting material and in the main fraction was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 9

Separation on Amberlite XAD-7, 40 °C, solvent system: water/ethanol				
Compound	Tea Concentrate (Starting material) of Example 6		Main Fraction of Example 6	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
Gallic acid	0.08	0.1	0	0.0
Catechin	0.51	0.6	3	1.0
Caffeine	9.48	11.3	4	1.4
EGCG	43.01	51.4	276	93.2
Epicatechin	4.34	5.2	9	3.0
GCG	8.23	9.8	2	0.7
ECG	18.03	21.5	2	0.7
Total	83.68	100.0	296	100.0

Example 7

[0049] The regenerated resin of Example 6 was equilibrated with a mixture of water/isopropanol (ratio 9:1 by volume)

[0050] 20 g of a concentrated catechin powder "Green tea extract, min. 95% of polyphenols" from Guizhou Highyin

Biological Products Co.(starting material) were dissolved in 20 ml of a mixture of water/isopropanol (ratio 9:1 by volume) by volume. Afterwards, 14 g of this solution (corresponding to 3.21 g of EGCG) were applied to the top of the column, and eluted with a mixture of water/isopropanol (ratio 9:1 by volume) with a constant flow rate of 18 ml/min at a column temperature of 60 °C. The eluent was degassed and kept under an nitrogen atmosphere prior to use. After an initial eluate of 1.35 l (prefraction), the flow rate was changed to 16.5 ml/min and a main eluate of 2.03 l was collected . The EGCG concentration in the main eluate was 0.998 g/l. With respect to other main catechins and caffeine, the purity of the epigallocatechin gallate in the main eluate determined by HPLC was 85.7 %. The yield based on EGCG was 62.8 %. During the experiment the pressure in the system varied from 1 to 2 bar depending on the flow rate applied.

[0051] To regenerate the resin, the remaining catechins were desorbed by eluting with 2.03 l of a mixture of water/isopropanol(ratio 4:6 by volume) at a flow of 16.5 ml/min and a temperature of 40° C. This fraction can also be used for further purification or separation of the desorbed catechins. Table 10 compares the concentration of the tea components in the eluate and in the starting material, thus illustrating the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the starting material and in the main fraction was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 10

Separation on Amberlite XAD-7, 60 °C, Solvent system: water/isopropanol				
Compound	Tea Concentrate (starting material) of Example 7		Main Fraction of Example 7	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
Gallic acid	0.05	0.1	0	0.00
Catechin	0.38	0.4	8	0.7
Caffeine	9.48	10.8	28	2.4
EGCG	45.42	51.8	998	85.7
Epicatechin	4.38	5.0	16	1.4
GCG	8.80	10.0	35	3.0
ECG	19.12	21.8	80	6.9
Total	87.63	100.0	1165	100.0

Example 8

[0052] The regenerated resin of Example 7 was equilibrated with a mixture of water/isopropanol (ratio 9:1 by volume).

[0053] 20 g of a concentrated catechin powder "Green tea extract, min. 95% of polyphenols" from Guizhou Highyin Biological Products Co.(starting material) were dissolved in 20 ml of a mixture of water/isopropanol (ratio 9:1 by volume). Afterwards, 14 g of this solution (corresponding to 3.10 g of EGCG) were applied to the top of the column. EGCG was eluted with a mixture of water/isopropanol (ratio 9:1 by volume) with a constant flow of 16.9 ml/min at a column temperature of 40 °C. The eluent was degassed and kept under an nitrogen atmosphere prior to use. After an initial eluate of 2.48 l (prefraction), the flow was changed to 23.66 ml/min and a main eluate of 4.95 l was collected. The EGCG concentration in the main eluate was 0.370 g/l. With respect to other main catechins and caffeine, the purity of the EGCG in the main fraction determined by HPLC was 86.4 %. The yield based on EGCG was 59.2 %. During the experiment the pressure in the system varied from 1 to 2 bar depending on the flow rate applied.

[0054] To regenerate the resin, the remaining catechins were desorbed by eluting with 2.03 l of a mixture of water/isopropanol (ratio 4:6 by volume) at a flow of 16.5 ml/min and a temperature of 40° C. This fraction can also be used for further purification or separation of the desorbed catechins.

Table 11 compares the concentration of the tea components in the eluate and in the starting material, thus illustrating the separation effect as shown by the relative percentage of EGCG. The concentration of the tea components in the starting material and in the main fraction was determined by HPLC using UV absorbance and expressed as wt.-% or ppm.

Tab. 11

Separation on Amberlite XAD-7, 40 °C, Solvent system: water/isopropanol				
Compound	Tea concentrate (starting material) of Example 8		Main fraction of Example 8	
	HPLC /wt.-%	Rel. Percentage /%	HPLC /ppm	Rel. Percentage /%
Gallic acid	0.20	0.2	0	0.0
Catechin	0.49	0.6	4	0.9
Caffeine	9.21	10.8	6	1.4
EGCG	43.74	51.5	370	86.4
Epicatechin	4.23	5.0	14	3.3
GCG	8.50	10.0	11	2.6
ECG	18.52	21.8	23	5.4
Total	84.89	100.0	428	100.0

Example 9 Separation of EGCG over Polyamide 11 using organic solvents

[0055] A commercially available green tea extract ("Green tea extract, min. 95 % of polyphenols", Lot#960328 from Guizhou Highyin Biological Product Co., Guiyang, China) containing catechins and caffeine in an amount as shown in Tab. 12 was used as the starting material. The concentration of the tea components was determined by HPLC using UV absorbance.

Tab. 12

Concentration of tea components in the starting material		
Compound	Tea extract HPLC /wt.-%	Tea extract Rel. Percentage /%
Gallic acid	0.01	0.0
EGC	2.02	3.0
Catechin	0.78	1.2
Caffeine	8.48	12.5
EGCG	36.87	54.5
Epicatechin	4.48	6.6
GCG	4.77	7.1
ECG	10.22	15.1
Total	67.63	100.0

[0056] 250 g of a commercially available Polyamide 11 (Cat. No. 1.07435.0100, origin Merck, Darmstadt, Germany) having a particle size of 5-40 microns were suspended in 300 ml ethyl acetate and transferred into a column having an inner diameter of 5 cm and a length of 36 cm. The column was equipped with a heating jacket and thermostat to 40°C. 3 g of the starting green tea extract, characterized in Tab. 12, containing 1.11 g of pure EGCG were dissolved in 153 ml of ethyl acetate and applied to the top of the column. An ethyl acetate/ethanol gradient elution (500 ml ethyl acetate, 1000 ml ethyl acetate/ethanol (8.5:1.5 v/v), 1000 ml ethyl acetate/ethanol (7:3 v/v), 2000 ml ethyl acetate/ethanol (1:1 v/v)) under a pressure of 0.3 bar afforded a main fraction of 550 ml which after evaporation of the solvents gave 1.12 g of solid containing 0.87 g EGCG as the main catechin component. The EGCG concentration in the main eluate was 0.186 %. The yield of separated EGCG calculated from 1.106 g EGCG present in the starting tea extract was 76 %.

[0057] To regenerate the resin, elution with 500 ml ethanol desorbed the remaining catechins. Before the next sep-

aration, the column was conditioned with 500 ml of ethyl acetate.

[0058] Table 13 illustrates the separation effect. The concentration of the tea components in the main eluate was determined by HPLC using UV absorbance.

Tab. 13

Concentration of tea components in the residue of the main eluate (after solvent evaporation)		
Compound	Residue of main fraction HPLC /wt.-%	Residue of main fraction Rel. Percentage /%
Gallic acid	0	0
EGC	0	0
Catechin	0	0
Caffeine	0	0
EGCG	77.40	96.4
Epicatechin	0.05	0.1
GCG	0.74	0.9
ECG	2.07	2.6
Total	80.26	100.0

Example 10 Separation of EGCG over Polyamide 11 using aqueous solvent mixture

[0059] An aqueous green tea extract solution containing catechins and caffeine in an amount as shown in Tab. 14 was used as the starting material. The concentration of the tea components was determined by HPLC using UV absorbance and expressed in wt.-%

Tab. 14

Concentration of the tea components in the residue of the starting tea extract solution (after solvent evaporation)		
Compound	Tea extract HPLC /wt.-%	Tea extract Rel. Percentage/%
Gallic acid	1.36	4.8
EGC	3.61	12.6
Catechin	1.45	5.1
Caffeine	6.89	24.1
EGCG	10.14	35.5
Epicatechin	1.59	5.6
GCG	0.99	3.5
ECG	2.51	8.8
Total	28.54	100.0

[0060] 25g of Polyamide 11 (Cat. No. 1.07435.0100, origin Merck, Darmstadt, Germany) having a particle size of 5-40 microns were suspended in 100 ml water and the pH adjusted to 6.5. This suspension was transferred into a column having an inner diameter of 3 cm and a length of 8 cm. 10 ml of the above green tea extract (Tab. 14) containing 0.078 g of pure EGCG were applied to the top of the column. A water/ethanol gradient elution (500 ml water, 600 ml water/ethanol (7:3 v/v), 350 ml water/ethanol (6:4 v/v), 500 ml water/ethanol (1:1v/v)) with a flow of 5 ml/min afford a main fraction of 110 ml (0.072 g) containing 0.046 g EGCG. The EGCG concentration in the main eluate was 0.06 %.

The yield of separated EGCG starting from 0.078 g EGCG in the tea extract was 59 %.

[0061] To regenerate the resin, elution with 500 ml ethanol desorbed the remaining catechins. Before the next separation, the column was conditioned with 500 ml of water.

[0062] Table 15 illustrates the separation effect. The concentration of the tea components in the main eluate was determined by HPLC using UV absorbance.

Tab. 15

Concentration of the tea components in the residue of the main fraction (after solvent evaporation)		
Compound	Residue of main fraction HPLC /wt.-%	Residue of main fraction Rel. Percentage /%
Gallic acid	1.10	1.6
EGC	0.00	0.0
Catechin	1.29	1.9
Caffeine	0.00	0.0
EGCG	63.53	91.7
Epicatechin	0.00	0.0
GCG	0.16	0.2
ECG	3.20	4.6
Total	69.28	100.0

Example 11 Separation of EGCG over Amberlite XAD-7, solvent system: water/ethanol

[0063] 416 ml Amberlite XAD-7 resin having a mean particle diameter having a particle diameter between 0.3 and 1.2 mm were filled into a laboratory chromatography column made from glass (ECO 25/999 M3V-K from Stagroma AG, Wallisellen, Switzerland) having an inner diameter of 2.5 cm and a length of 100 cm. The column was equipped with a heating jacket and thermostatted to 60°C. The resin was washed and equilibrated with a mixture of water/ethanol (ratio 9:1 by volume).

A commercially available green tea extract ("Tea polyphenols TP-80" from Zhejang Zhongke Plant Technical Co. Ltd., Hangzhou, Zhejang, P. R. China.) containing catechins and caffeine in an amount as shown in Tab. 16 was used as the starting material. The concentration of the tea components was determined by HPLC using UV absorbance and expressed in wt.-%.

Tab. 16

Concentration of tea components in the starting material		
Compound	Tea extract of Example 13 HPLC /wt.-%	Tea extract of Example 13 Rel. Percentage /%
Gallic acid	0.1	0.1
EGC	8.6	10.1
Catechin	1.9	2.2
Caffeine	6.2	7.3
EGCG	40.3	47.4
Epicatechin	10.4	12.2
GCG	0.9	1.1
ECG	16.6	19.5
Total	85.0	100.0

[0064] 11.2 g of the starting green tea extract, characterized in Tab. 16, containing 4.5 g of pure EGCG were dissolved in 112.5 ml of deionized water and applied to the top of the column. The catechins were eluted with a mixture of water/ethanol (ratio 9:1 by volume) with a constant flow of 0.6 l/hour at a column temperature of 60 °C. The eluent was degassed and kept under an nitrogen atmosphere prior to use.

[0065] After an initial eluate of 1.2 l, the composition of the eluent was changed to water/ethanol 8:2 by volume. This elution with a total amount of 1.5 l afforded a main fraction of 900 ml containing 2.115 g EGCG. The EGCG concentration in the main fraction was 0.245 %. The yield of separated EGCG starting from 4.5 g EGCG in the tea extract was 47 %. During the experiment the pressure in the system varied from 0.8 to 1.5 bar.

[0066] To regenerate the resin, the elution was continued with a mixture of water/ethanol 4:6 by volume, thus ethanol desorbing the remaining catechins. Before the next separation, the column was conditioned with water/ethanol 9:1 by volume.

[0067] Table 17 illustrates the separation effect. The concentration of the tea components in the residue of the main fraction (after evaporation of the solvent) was determined by HPLC using UV absorbance and expressed as wt.-%.

Tab. 17

Concentration of tea components in the residue of the main fraction (after solvent evaporation)		
Compound	Residue of main fraction of Example 13 HPLC /wt.-%	Residue of main fraction of Example 13 Rel. Percentage /%
Gallic acid	0	0
EGC	0	0
Catechin	0.6	0.7
Caffeine	0.3	0.3
EGCG	81.4	94.5
Epicatechin	1.7	2.0
GCG	0.2	0.2
ECG	1.9	2.2
Total	86.1	100.0

Claims

1. A process for producing epigallocatechin gallate (EGCG), which comprises the steps of

   a) providing a green tea extract;
   b) subjecting the green tea extract to a chromatography on any macroporous polar resin commonly used for adsorption or chromatography at a temperature in the range of about 10° C to about 80° C;
   c) eluting EGCG from the macroporous polar resin with a polar elution solvent at a temperature in the range of about 10° C to about 80° C and at a pressure in the range of about 0.1 bar to about 50 bar;
   d) optionally concentrating the eluate of step c);
   e) optionally regenerating the macroporous polar resin by desorbing the remaining catechins; and
   f) optionally concentrating the desorbed catechins of step e).

2. A process according to claim 1 wherein the macroporous polar resin is selected from the group consisting of polyacrylates, polymethyacrylates polyamides and polyesters.

3. A process according to claim 2 wherein the polyacrylate resin is AMBERLITE ®XAD-7.

4. A process according to claim 2, wherein the polymethacrylate resin is AMBERCHROM ® CG-71.

5. A process according to claim 2, wherein the polyamide resin is Polyamide 11.

6. A process according to any one of claims 1 to 4, wherein steps b) and c) are carried out at a temperature in the range of about 20° C to about 60° C.

7. A process according to any one of claims 1 to 5, wherein step c) is carried out under a pressure of about 0.1 bar to about 20 bar, preferably of about 0.1 bar to about 10 bar.

8. A process according to any one of claims 1 to 6, wherein the polar elution solvent is a mixture of water and an organic solvent.

9. A process according to claim 7, wherein the elution solvent is a mixture of about 70 vol% to about 95 vol%, preferably about 90 vol%, of water and about 5 vol% to about 35 vol%, preferably about 10 vol%, of an organic solvent.

10. A process according to claim 7 or 8, wherein the organic solvent is ethanol, isopropanol, ethyl acetate or acetone.

11. A process according to any one of claims 1 to 9, wherein the flow rate of the elution solvent is in the range of about 0.5 to about 20 bed volumes/h, preferably about 0.5 to about 10 bed volumes/h, more preferably of about 0.8 to about 5 bed volumes/h.

12. A process according to any one of claims 1 to 10, wherein step d) is carried out by adding citric acid, ascorbic acid or isoascorbic acid.

13. A process according to claim 11, wherein the acid is added in an amount of about 0.1 to about 2.5 % with respect to EGCG.

14. A process according to any one of claims 1 to 12, wherein step e) is carried out by using a pure organic solvent or a mixture of about 10 to about 60 vol% of water and of about 40 to about 90 vol% of organic solvent, preferably about 40 vol% of water and about 60 vol% of organic solvent.

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(54) Process for the production of epigallocatechin gallate

(57) Epigallocatechin gallate (EGCG) is obtained by subjecting a green tea extract to chromatography on a macroporous polar resin; elating EGCG from the resin with a polar elution solvent; optionally concentrating the eluate; optionally regenerating the resin by desorbing the remaining catechins; and optionally concentrating the desorbed catechins.

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EUROPEAN SEARCH REPORT

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	26 January 2001	Francois, J

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